

# Mechanical culture conditions effect renal cell gene expression

## ISS Utilization-2001

Tulane Environmental

Astrobiology Lab

October 16, 2001

# Flight experiment

- To characterize renal cell gene expression changes during space flight
- to dissect gene expression changes due to vibration, gravity of launch and compare to rotating wall vessel
- to predict conditions to make bioproducts



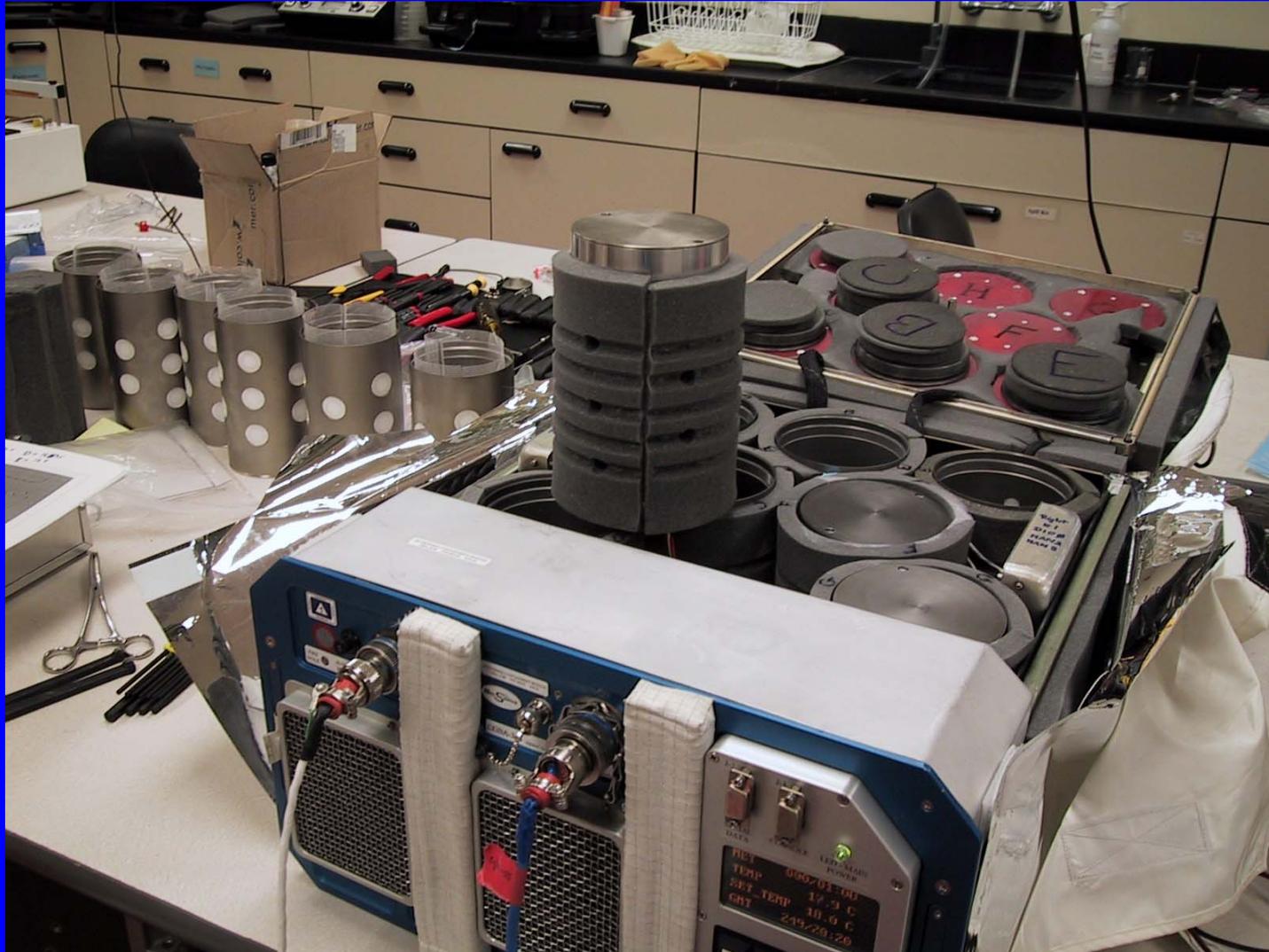
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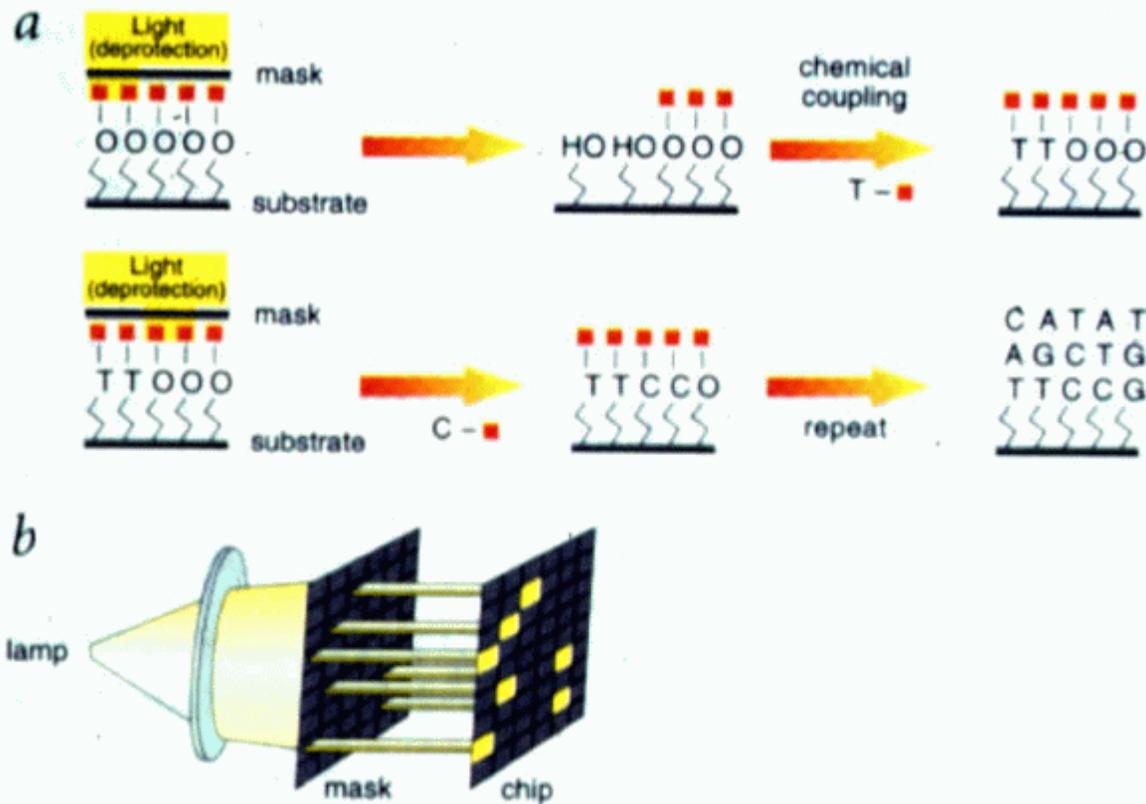


# Rotating wall vessel



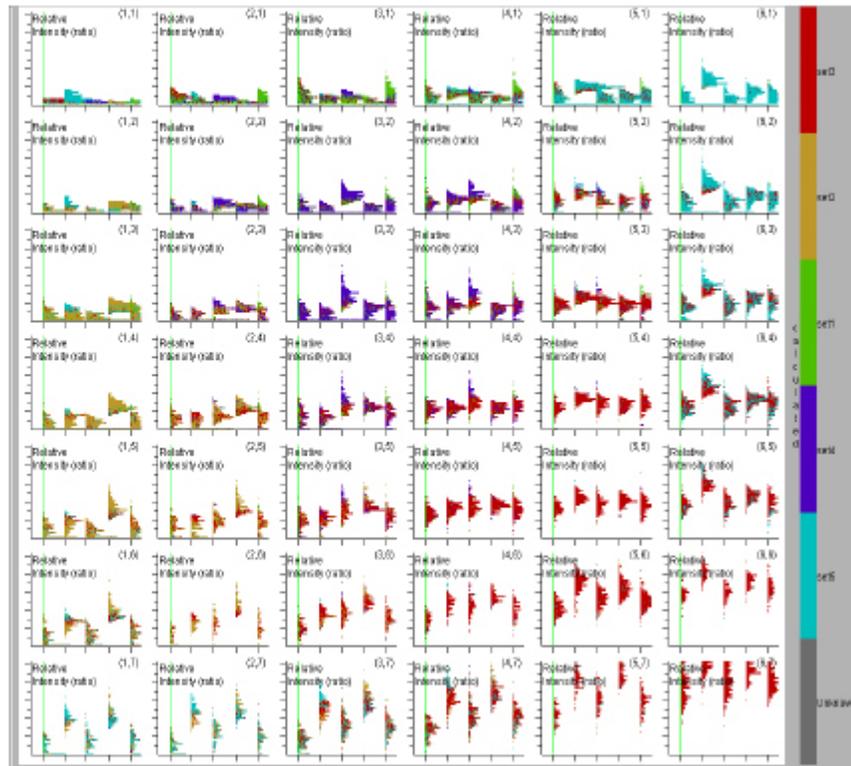
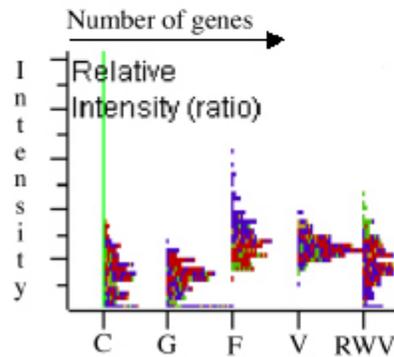
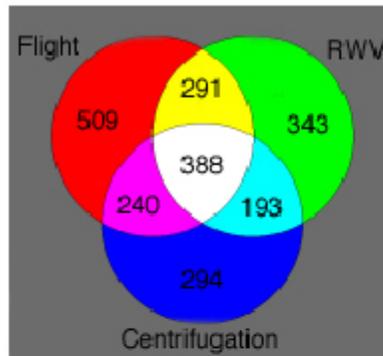
# BIOSERVE

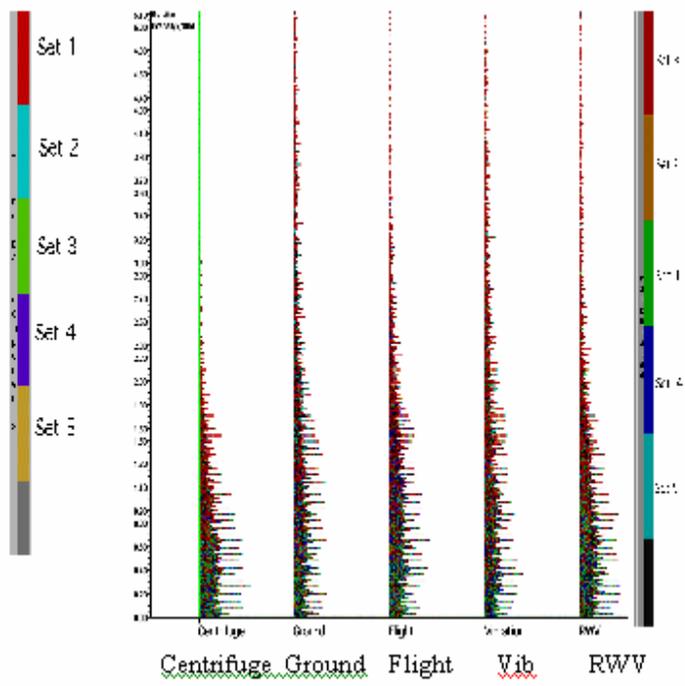
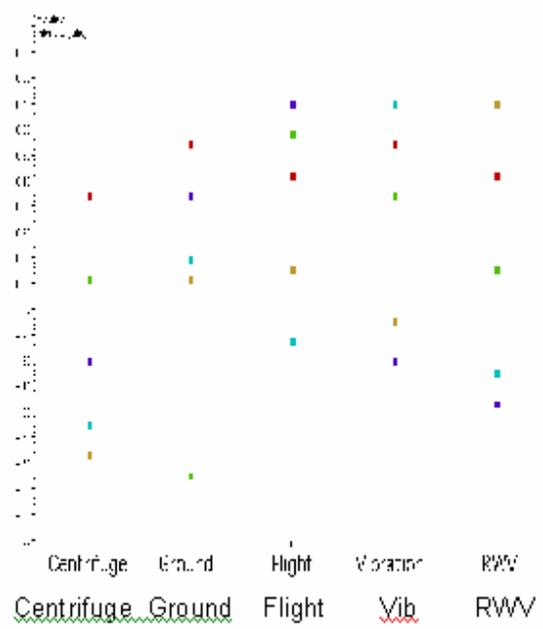
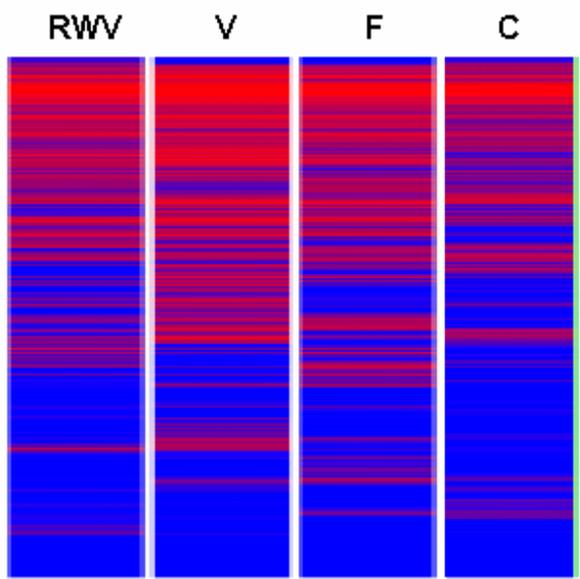




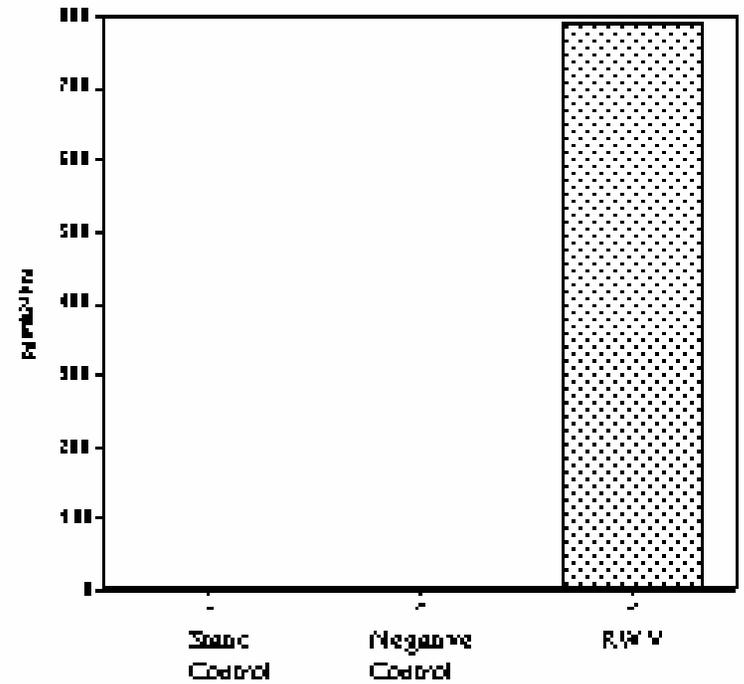
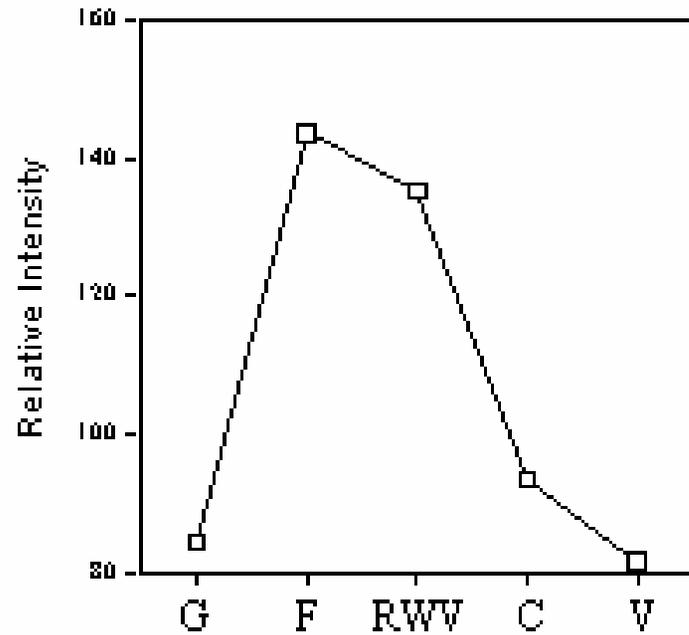
**Fig. 1 a**, Light directed oligonucleotide synthesis. A solid support is derivatized with a covalent linker molecule terminated with a photolabile protecting group. Light is directed through a mask to deprotect and activate selected sites, and protected nucleotides couple to the activated sites. The process is repeated, activating different sets of sites and coupling different bases allowing arbitrary DNA probes to be constructed at each site. **b**, Schematic representation of the lamp, mask and array.

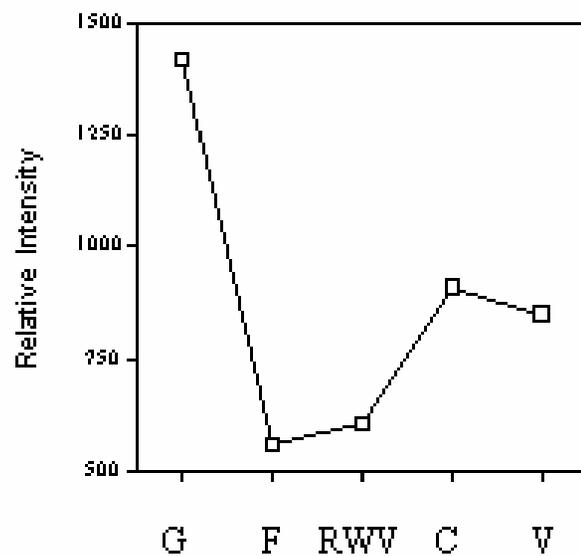
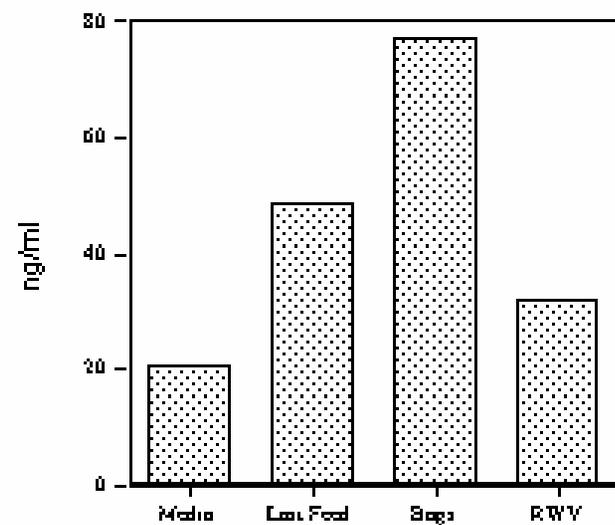
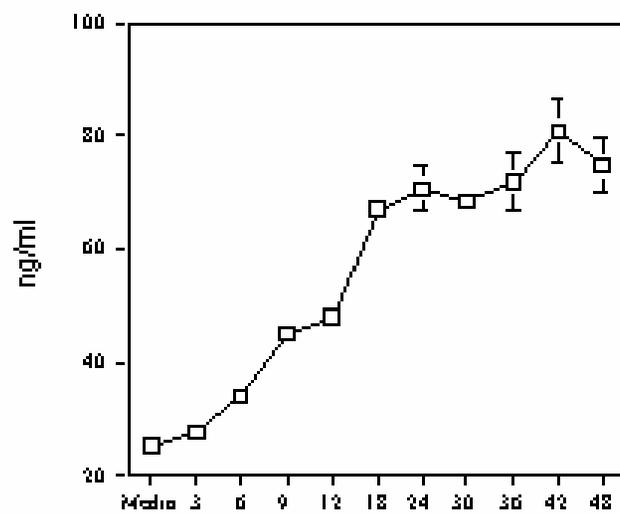
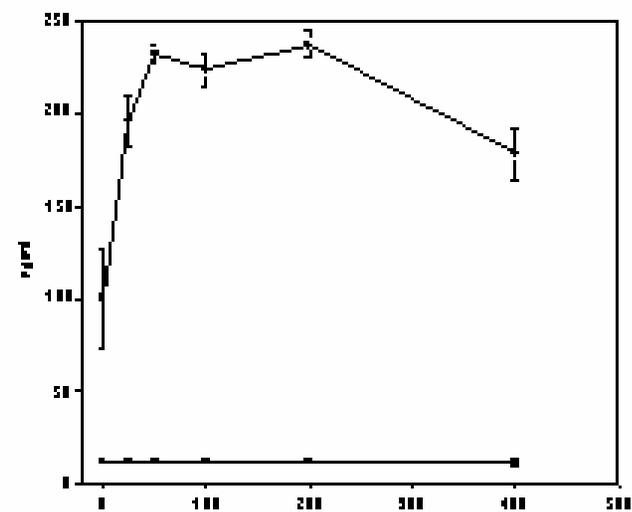
# Gene array clusters



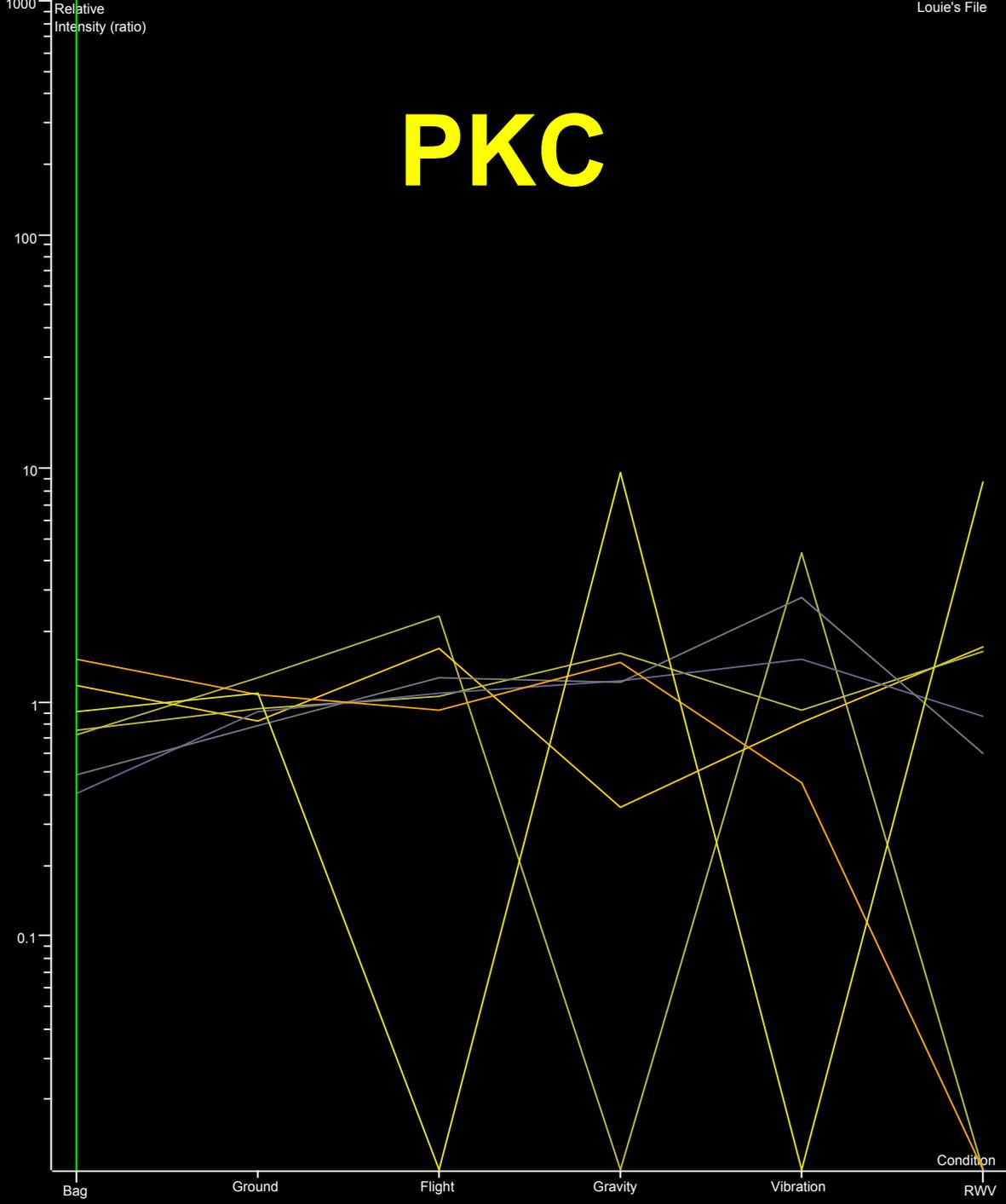


### 1 $\alpha$ -Hydroxylase Gene Expression



**A****B****C****D**

# PKC



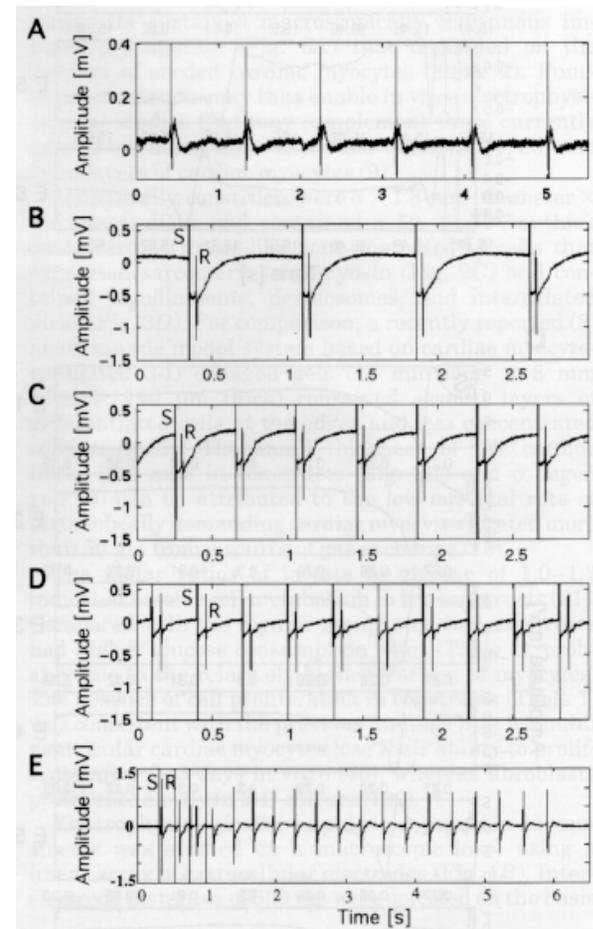
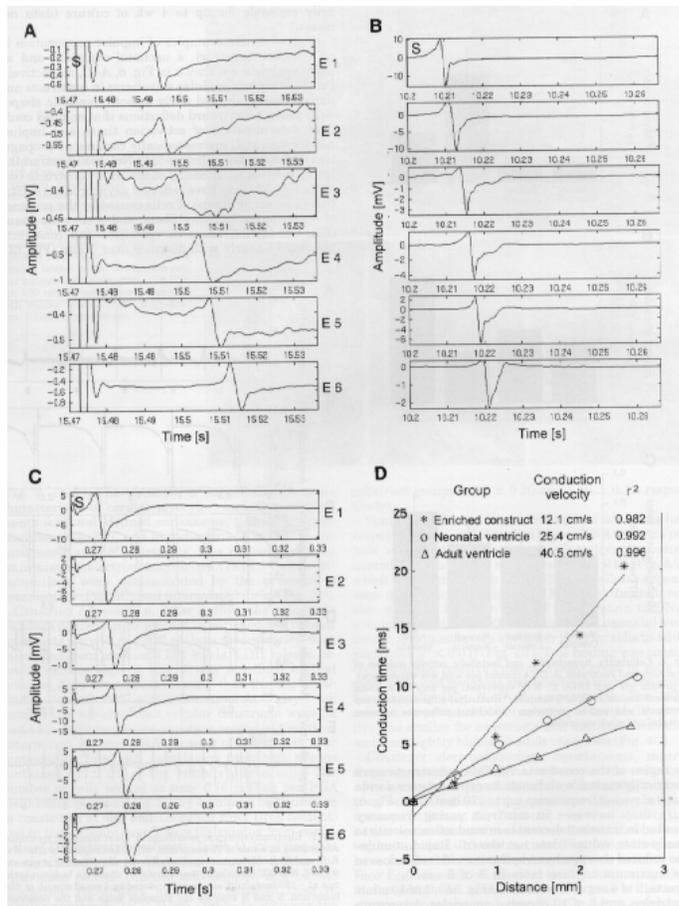
# Bioproduct production conditions

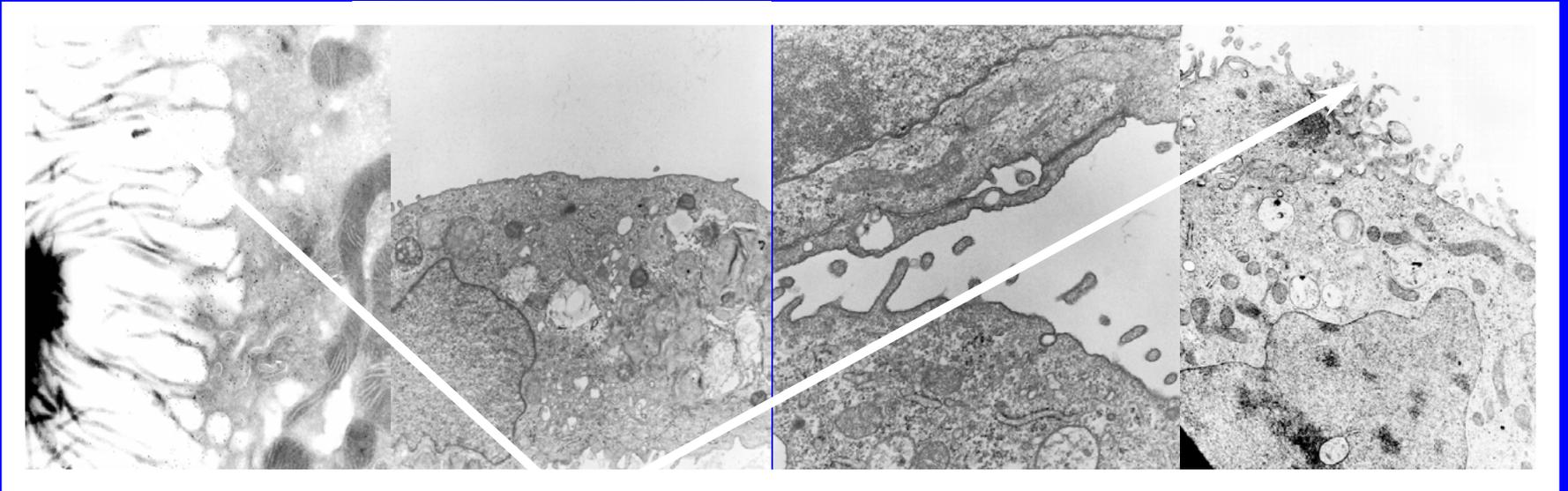
- Vitamin D : rotating wall vessel
- Interferon in flight
- Other - in intellectual property review

# Thanks - lab

- **Our lab**
- Pat Allen
- Ed Benes
- Luis Cubano
- Bryan Klassen
- **Linda Hyman's Lab**
- Linda Hyman
- Kelly Johansen
- **Paris - INSERM**
- Pierre Verroust
- Renata Korynsi
- **NASA**
- Tom Goodwin
- John Love
- Tacy Baker
- Lynn Harper

# Engineered Heart Tissue

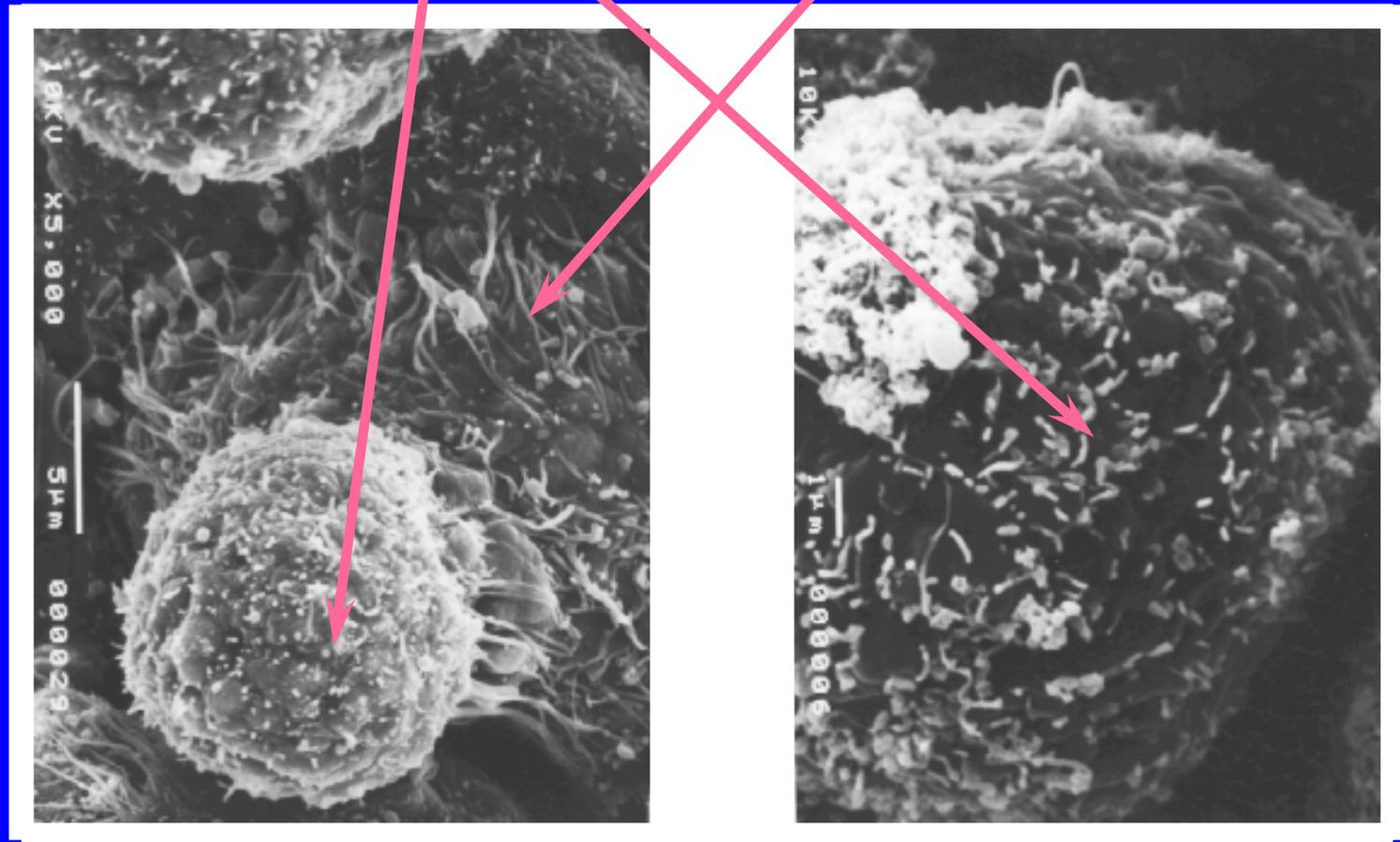




Microvilli with receptors for aminoglycosides  
Synthesis of Vitamin D precursors

# Production of Liver Tissue

Cellular shape ( ) and arrangement ( ) enabling investigation of infection in a more native tissue model

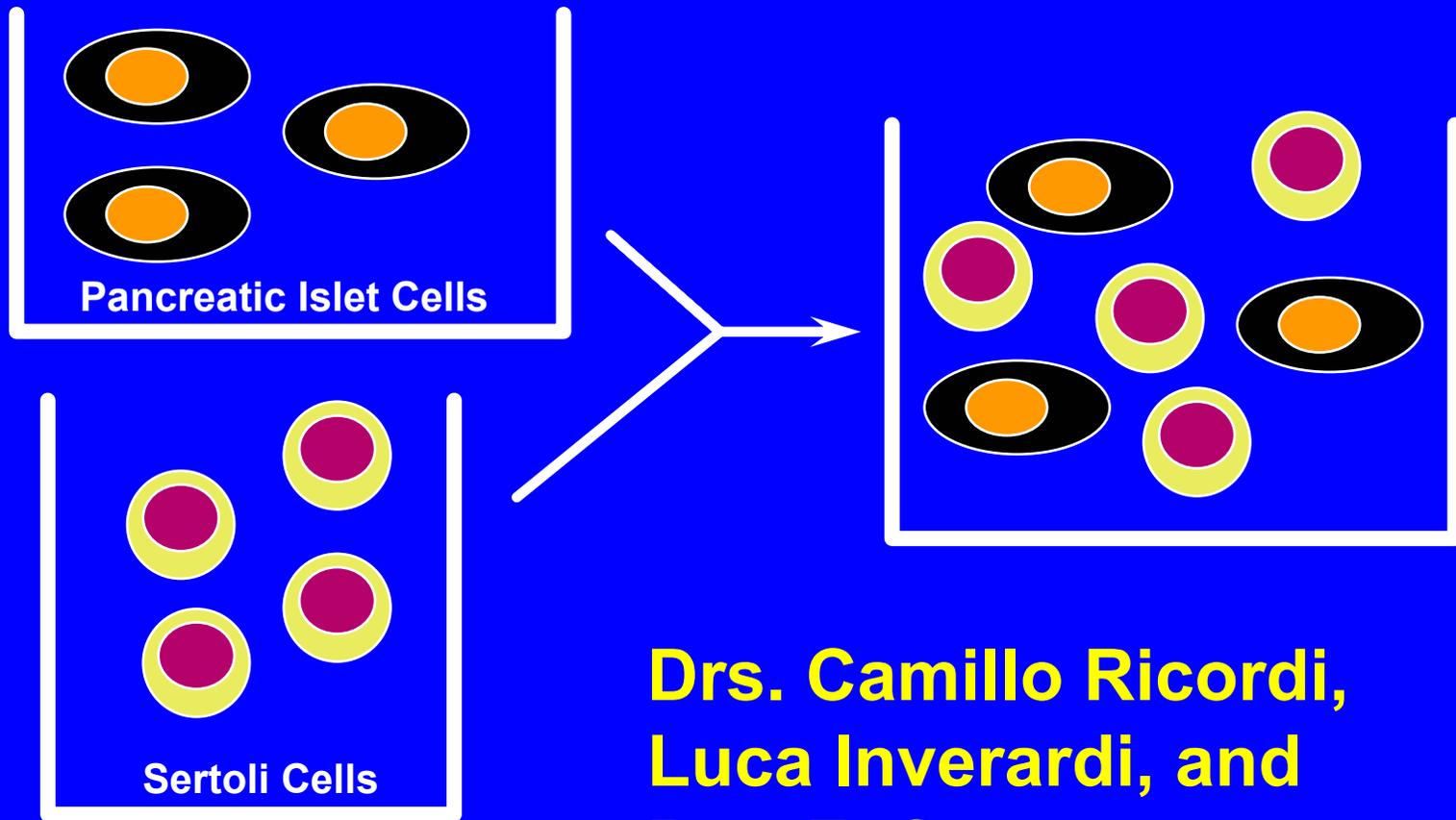


# Liver Tissue Morphogenesis

- Hepatitis virus pathogenesis model (BCM)
- Drug metabolism (StelSys, Inc.)
- Toxicology testing
- Extracorporeal liver support technology (StelSys, Inc.)

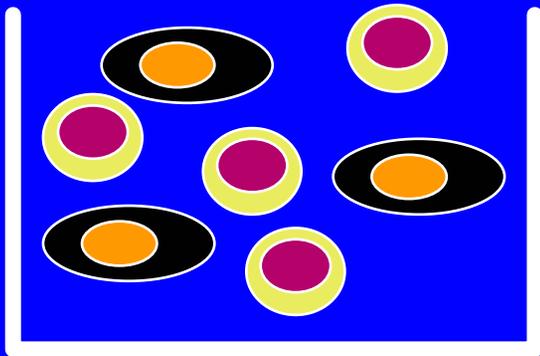
# Diabetes Treatment Models

## *Co-transplantation*

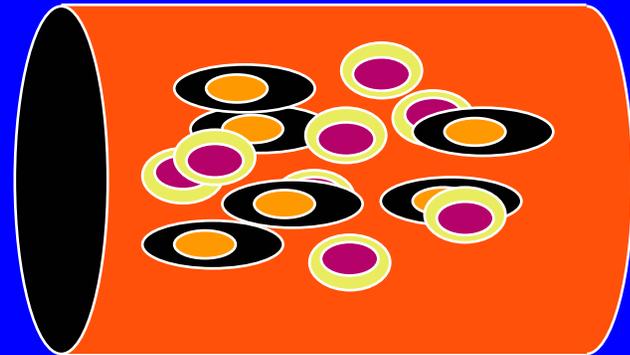


**Drs. Camillo Ricordi,  
Luca Inverardi, and  
Don F. Cameron**

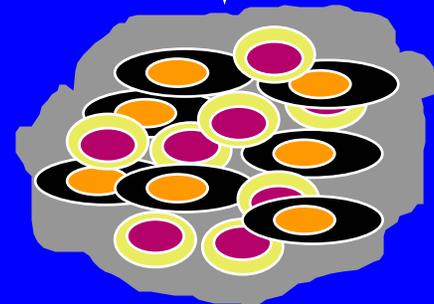
# Diabetes Treatment Model



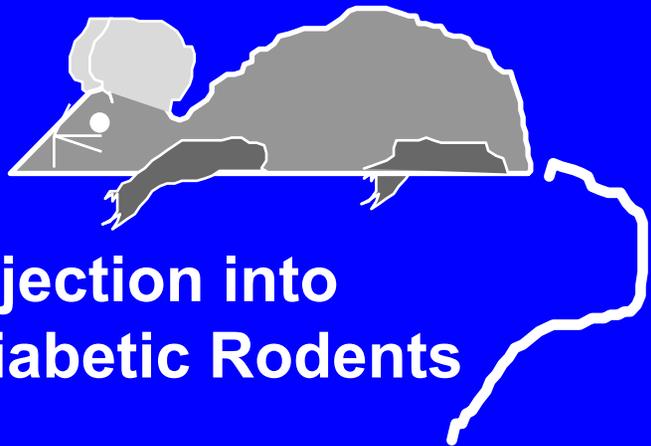
Mixed Culture



NASA Bioreactor



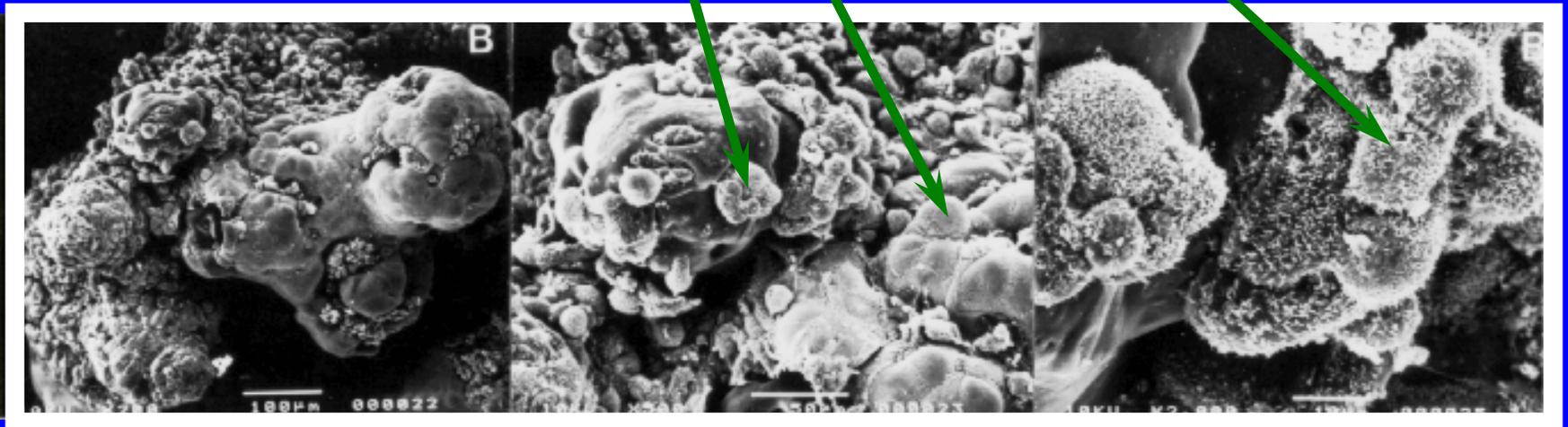
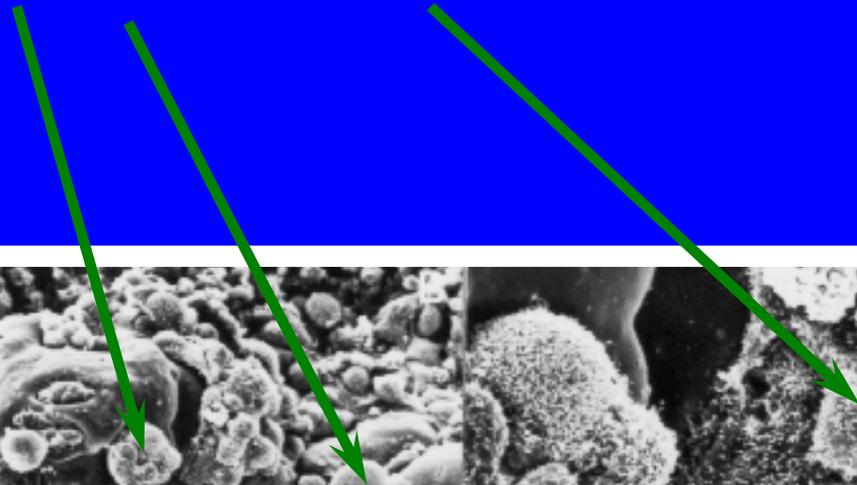
Immunoresistent Assembly



Injection into Diabetic Rodents

# Dynamic Models in Cancer Research

Human Immune Cells  
Invading Three Dimensional  
Breast Cancer from the Bioreactor



# Human Colon Cancer In Analog Culture

**Stationary  
Culture**

Monolayer

**Bioreactor  
3-D Culture**

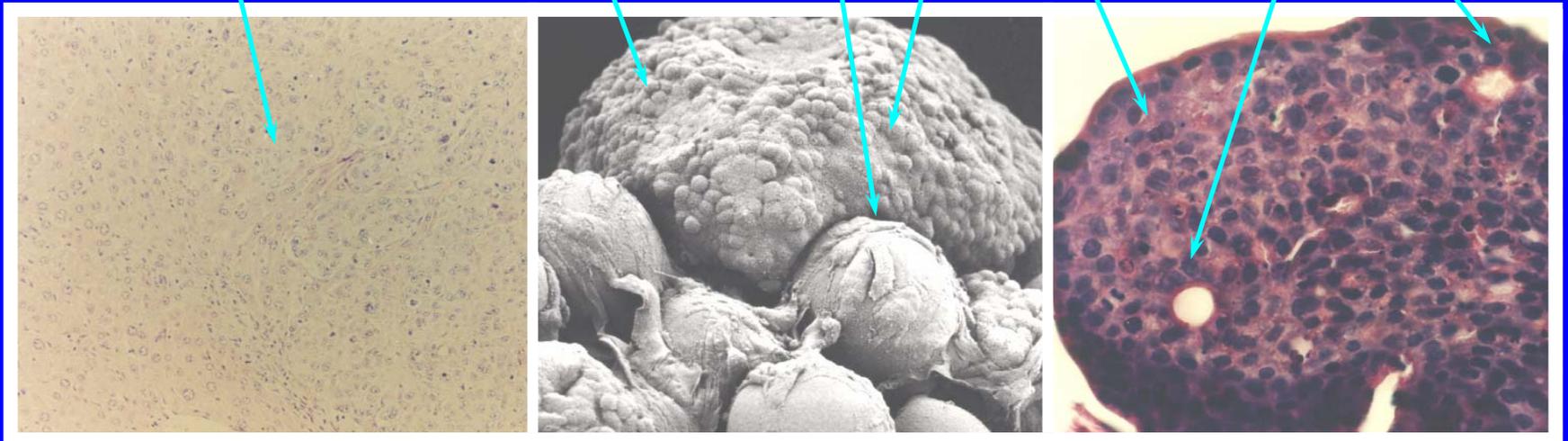
Polypoid  
Structure

Fibroblasts

Cancer Cells

**Bioreactor  
3-D Culture  
Histology**

Gland-like  
Structures

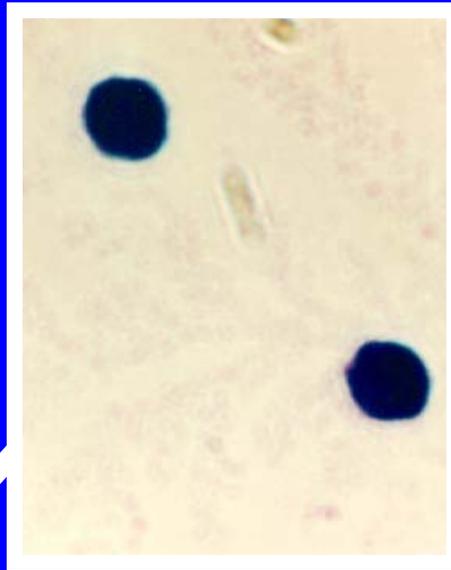
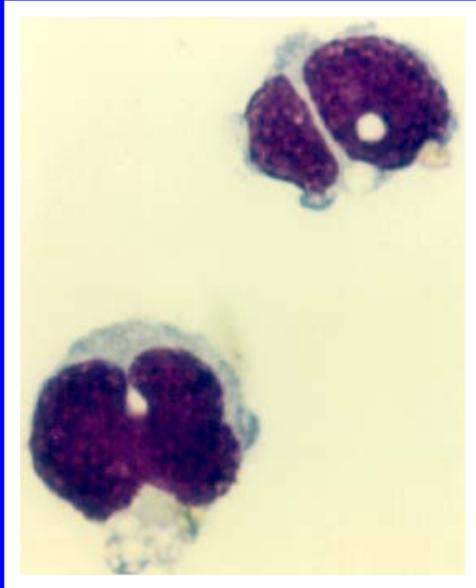


# Differentiation Model

## HL-60 Promyelocytic Leukemia Differentiation

**Bioreactor**

Monocyte  
CD14+ CD11b +



**Static**

CD14- CD11b -

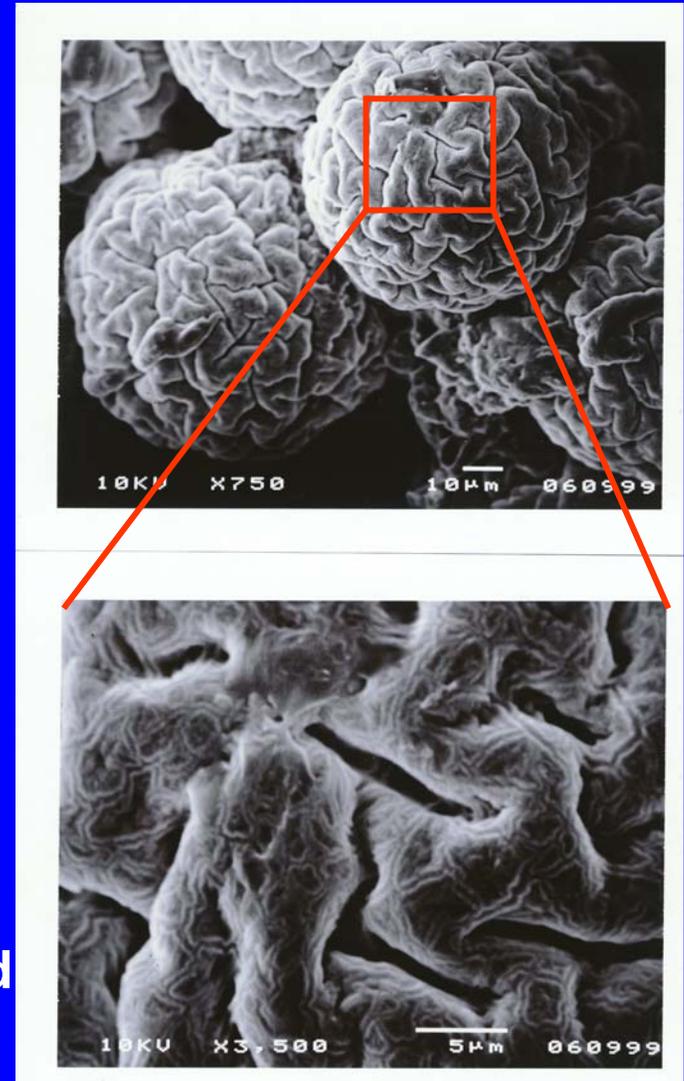


# Propagation of 'Difficult' Cells

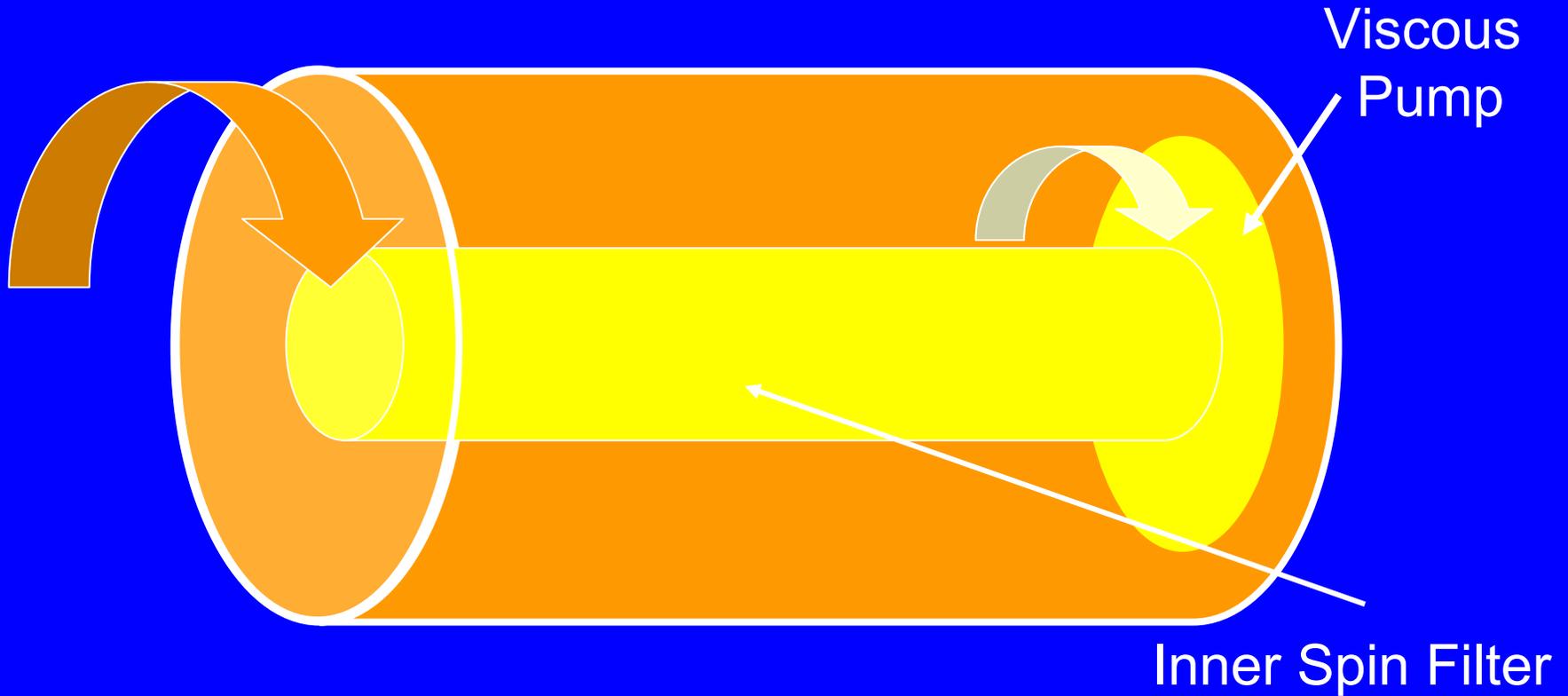
The top photo show the first continuous cultures of Bowhead whale kidney cells growing on spherical microcarriers from the NASA bioreactor.

The lower photo is a magnification of the indicated area showing the unique features of the tissues from this species.

Scientists will use these cells to investigate the response of mammals to environmental toxins in marine ecosystems. These toxins can find their way into the food chain not only affecting food sources but also the end user, humans.

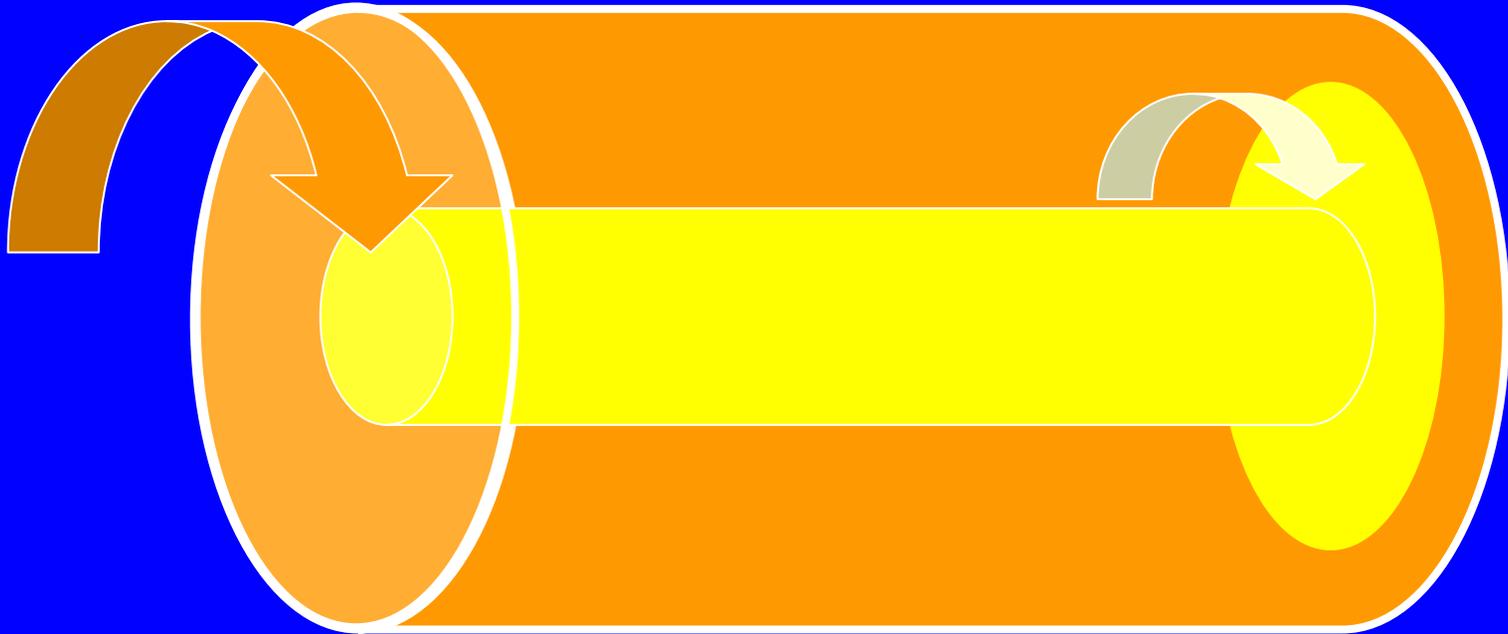


# Flight Bioreactor

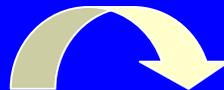


Can operate with coupled or differentially rotated

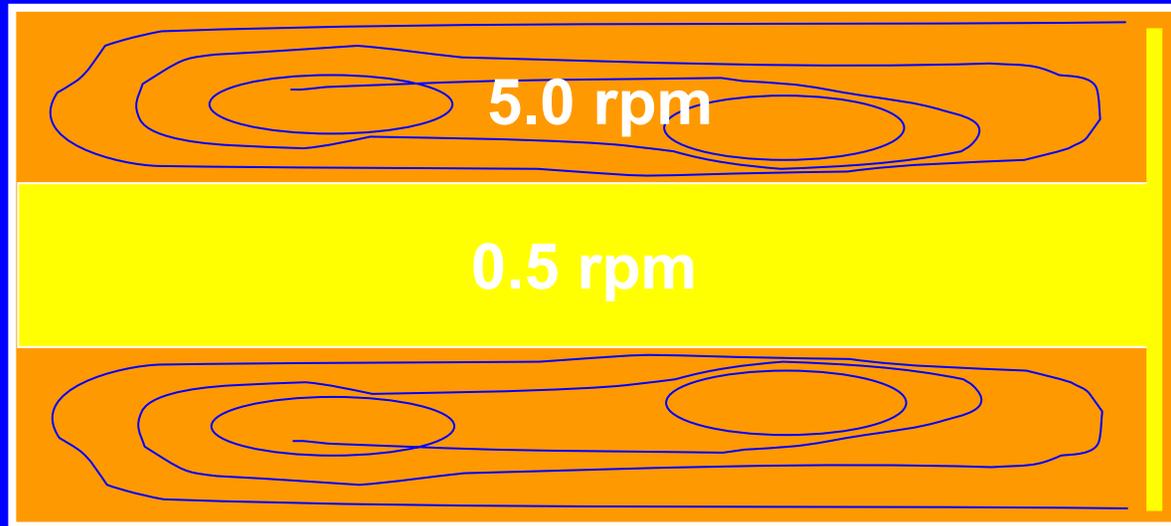
# Flight Bioreactor



 = 5 rpm

 = 0.5 rpm

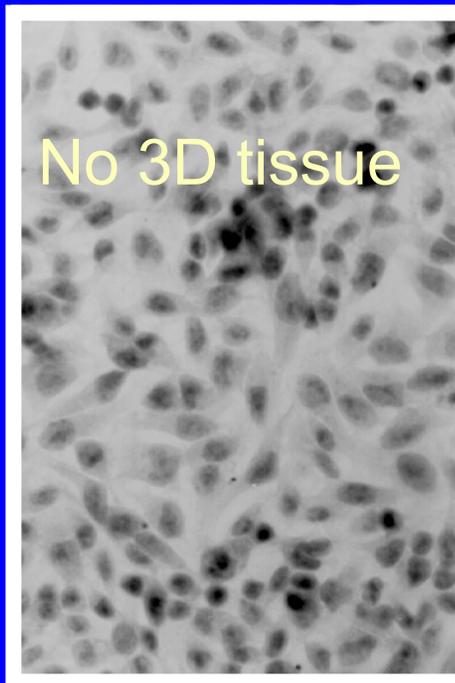
# Flight Bioreactor



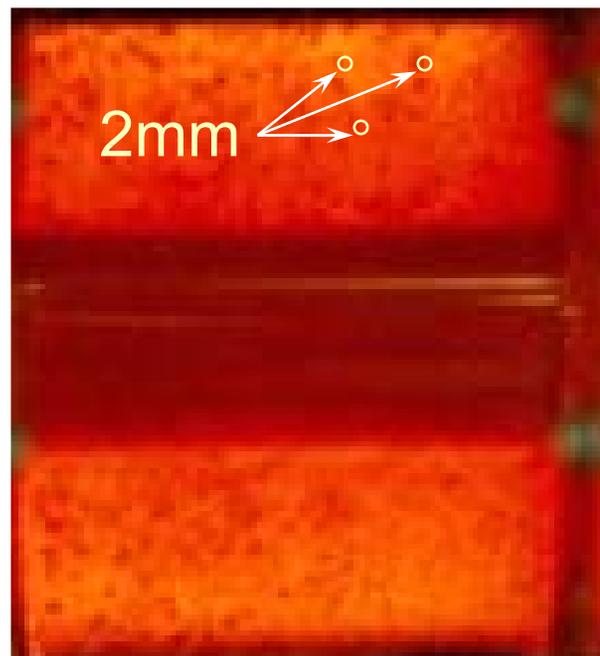
Differential Rotation sets a gentle flow pattern that maintains mass transfer of nutrients in a low shear environment ( $<0.05$  dynes/cm<sup>2</sup>)

# Comparison of Colon Cancer Cell Cultures

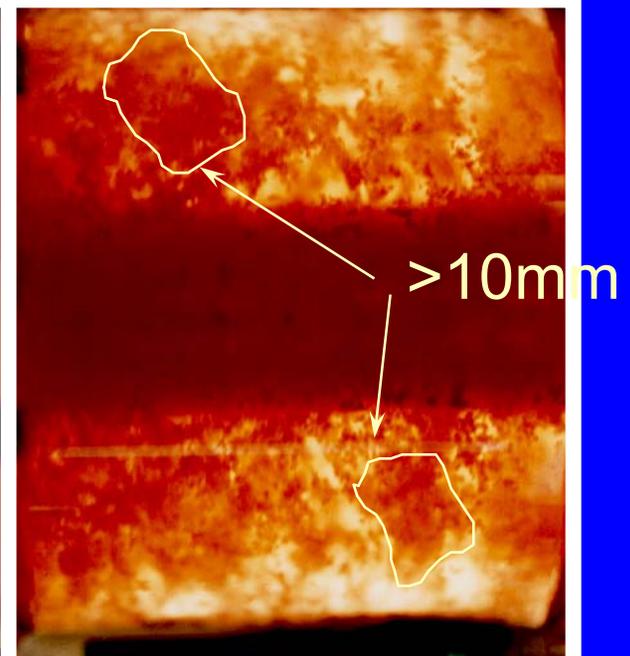
**Standard  
Monolayer  
Culture**



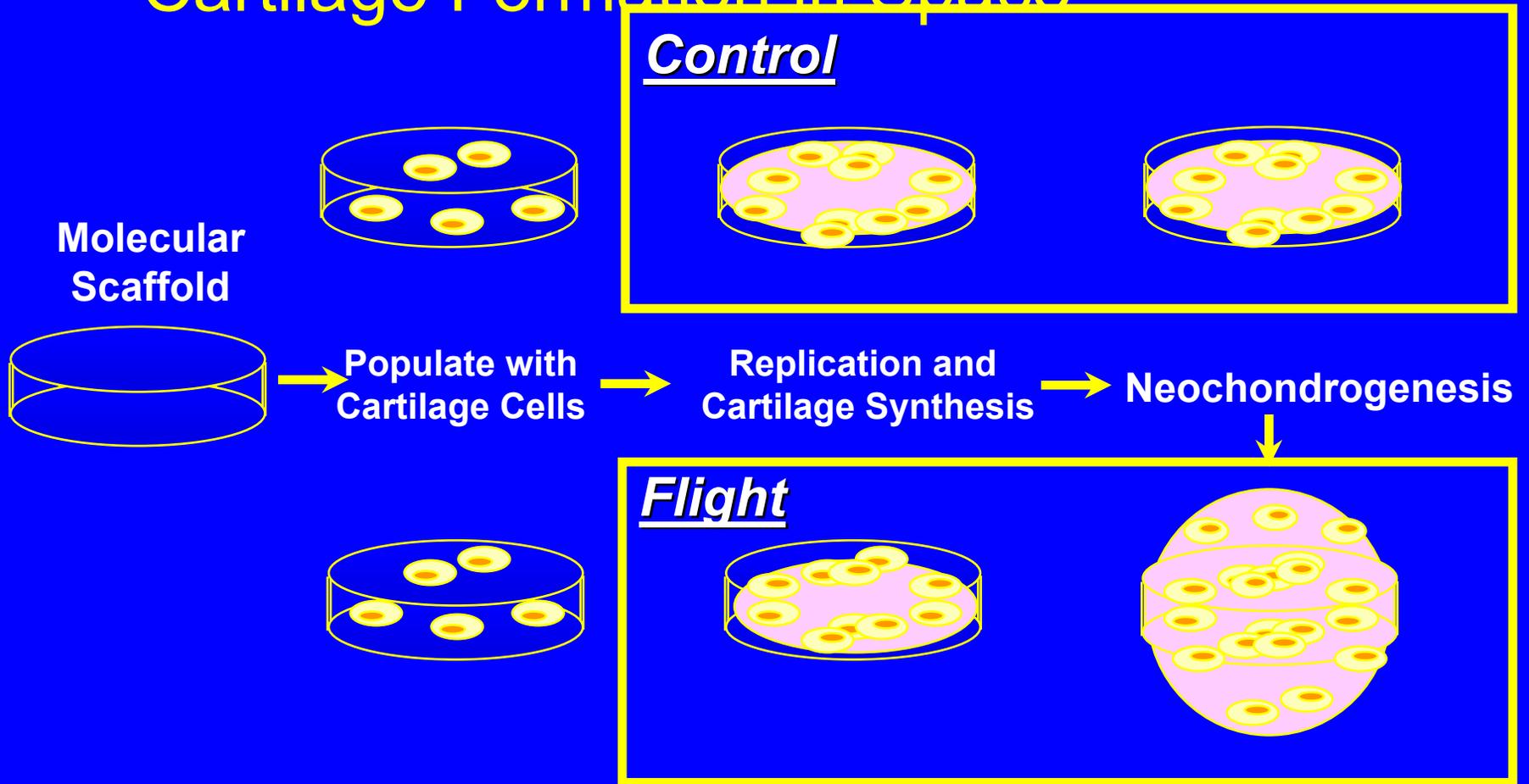
**Ground-Based  
Bioreactor Cell  
Culture**



**Space Bioreactor  
Cell Culture**

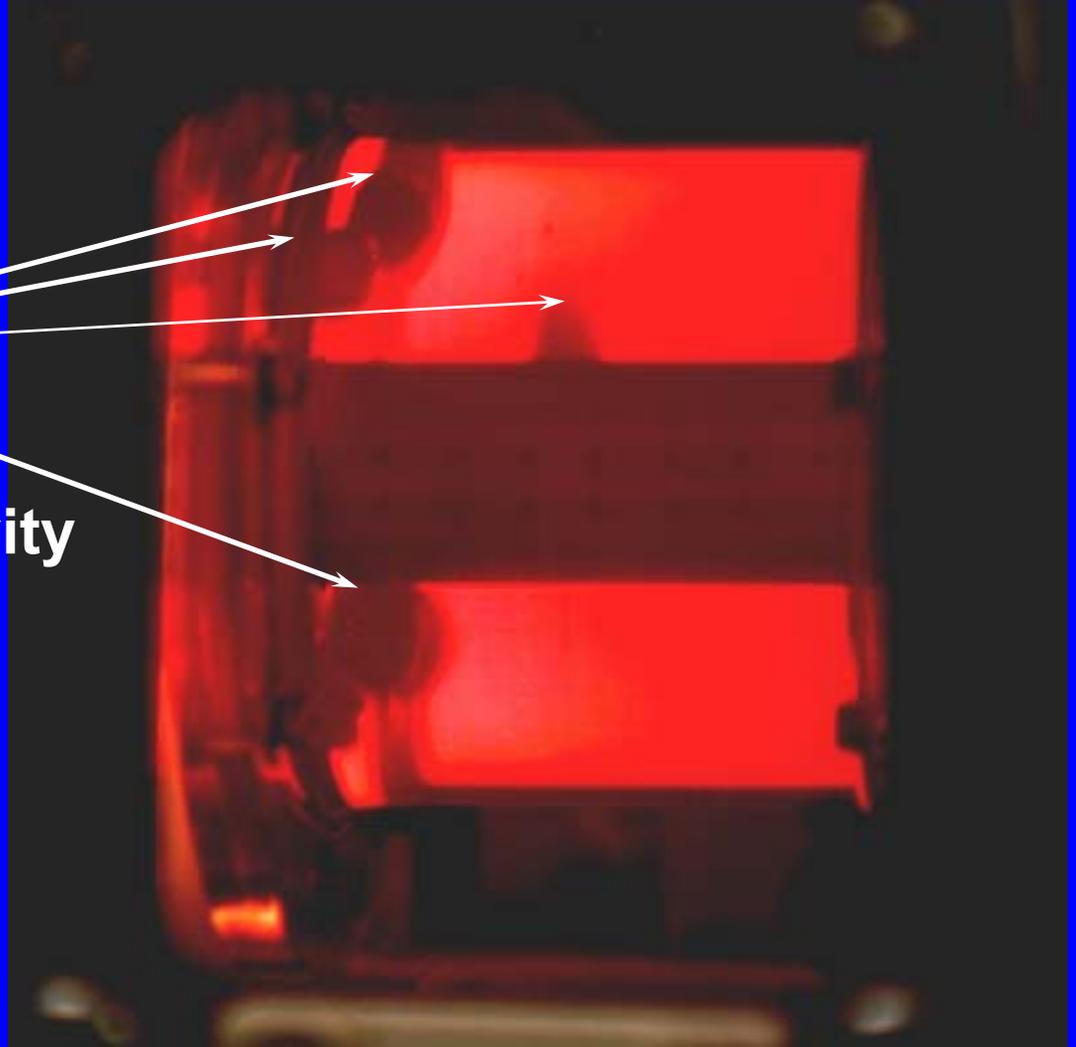


# Cartilage Formation in Space



# NASA Mir Increment 4

**Cartilage  
Construct after  
5 days in microgravity**



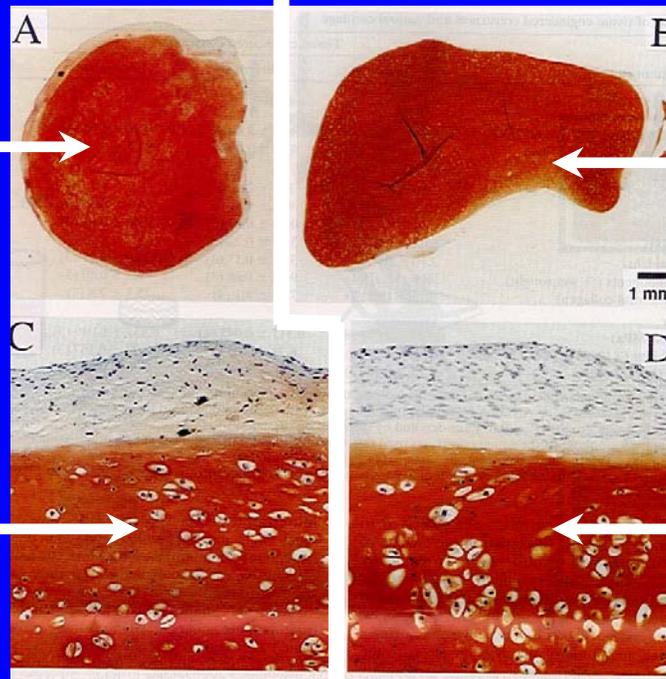
# Chondrogenesis in Microgravity

**NASA-Mir  
Bioreactor**

**Ground Based  
Bioreactor**

**Spherical  
Less Dense  
Immature  
Cartilage**

**Disk shaped  
More rigid  
Cartilage**



**Mechanically  
weak but  
compressible**

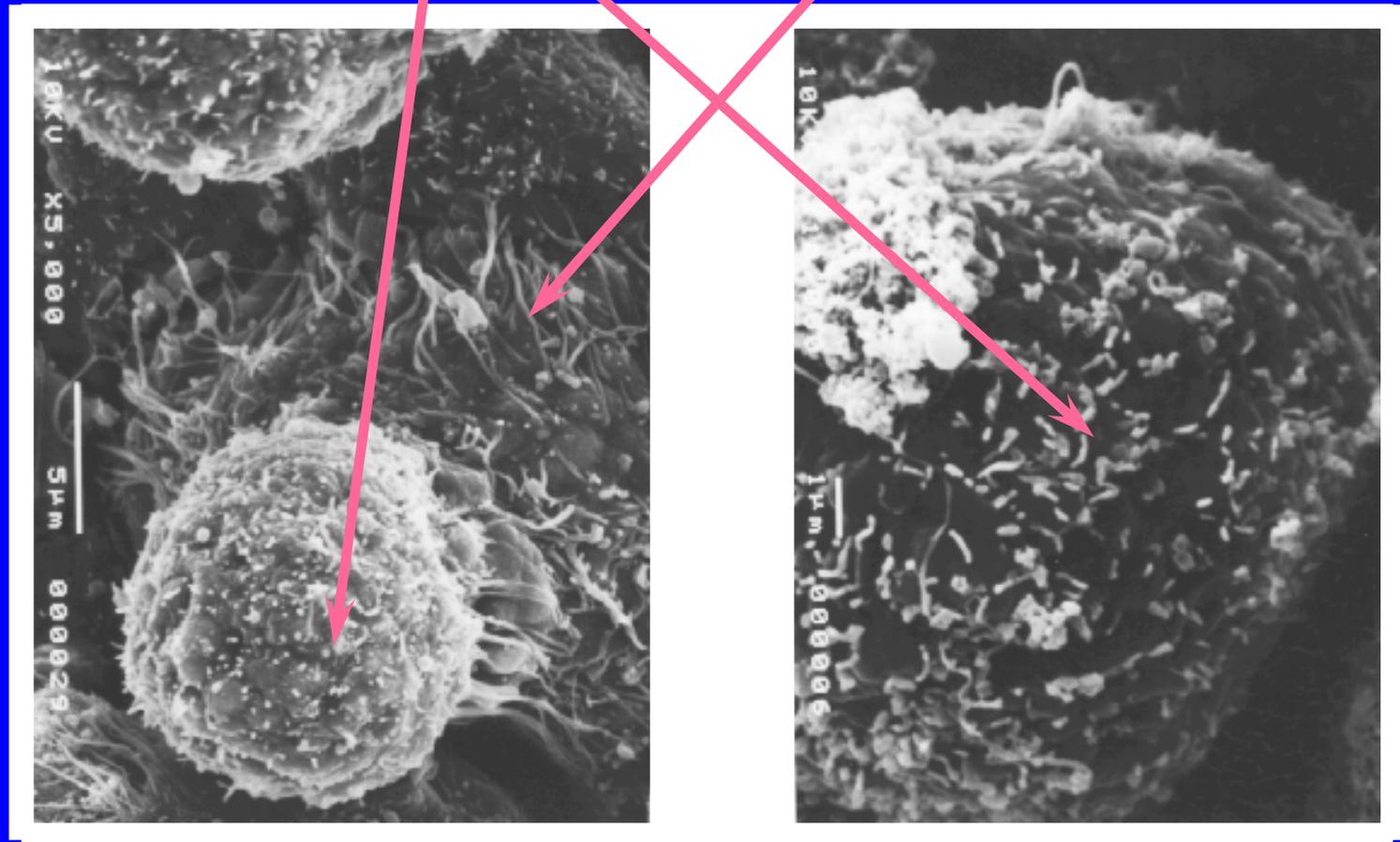
**Mechanically  
stronger and less  
compressible**

**Conclusion: Space cartilage is substantially different than mature native cartilage. The space and the ground based bioreactor cartilage formation are superior to that from standard culture techniques.**

*Freed*

# Production of Liver Tissue

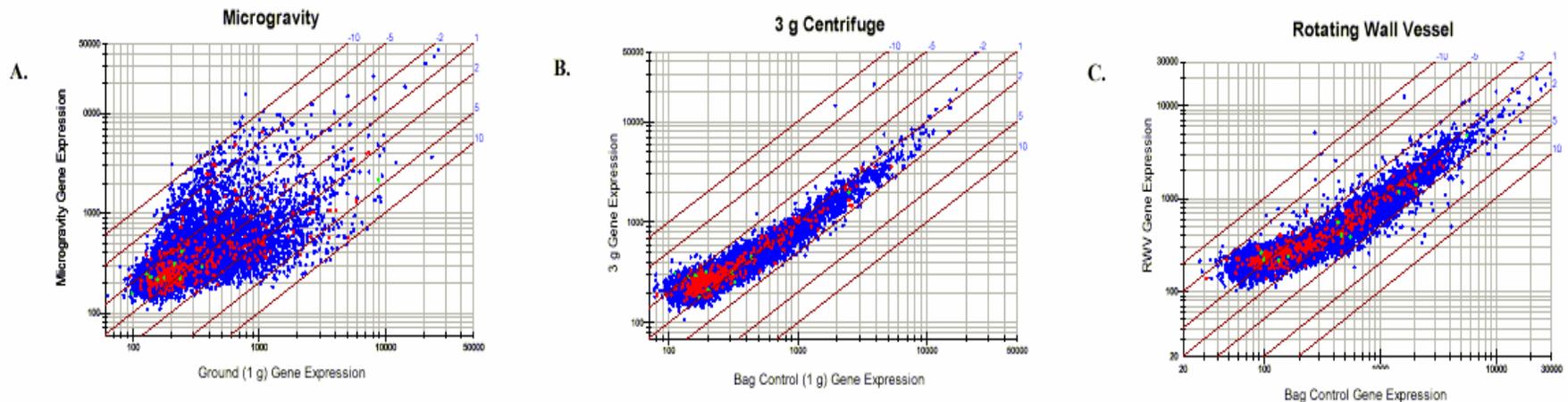
Cellular shape ( ) and arrangement ( ) enabling investigation of infection in a more native tissue model



# Liver Tissue Morphogenesis

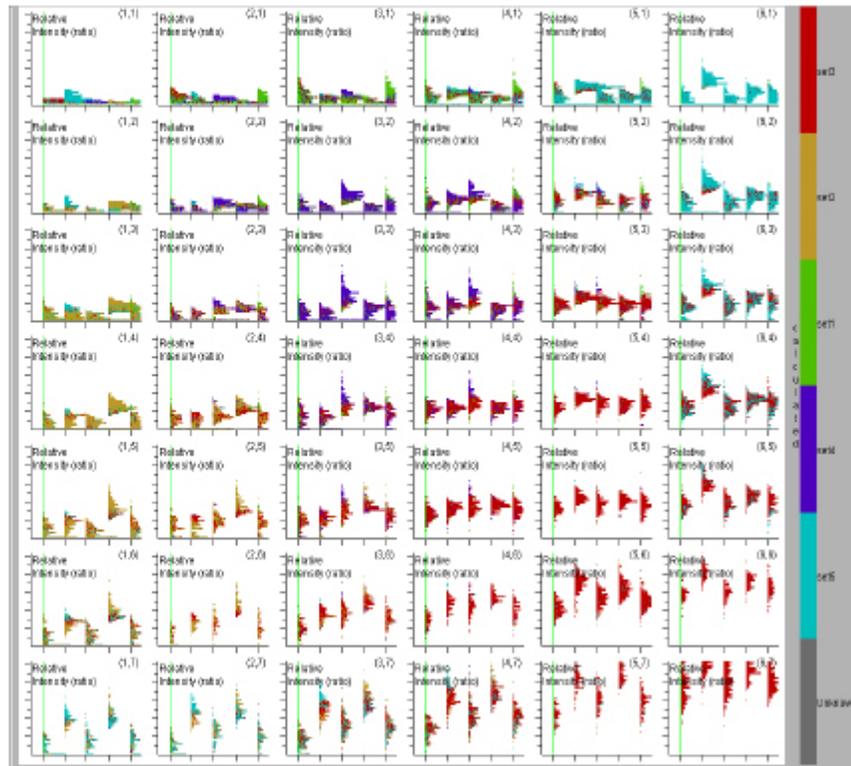
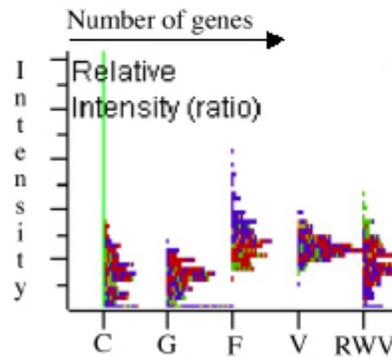
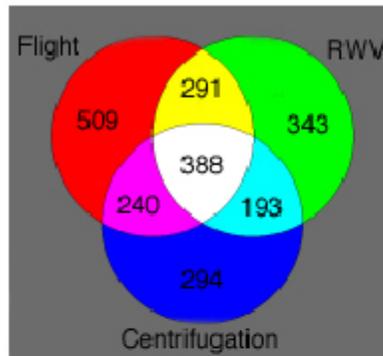
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- Drug metabolism (StelSys, Inc.)
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# Gene Expression in Microgravity



- The response of human cells to microgravity is fundamental to the adaptation of terrestrial life to low gravity environments and is reflected in the genes that are activated in space.
- As we observe the changes in cells in microgravity we open new opportunities into the fundamental processes in cells.
- Some of the arenas where the scientific community uses these findings is in the engineering of tissues for transplantation, modeling of disease, and the propagation of infectious organisms
- In May of 1999 Dr. Tim Hammond of Tulane University published his findings from microgravity and bioreactor experiments on the genes that are turned on and off when cells are transitioned to space ( Nature Medicine 5/99)

# Gene array clusters



# Space versus analog

- Decreased compared to Analog
  - Adhesion molecule
  - Apoptosis
  - Cytoskeletal proteins
  - Differentiation
  - Intracellular signaling proteins
  - Receptors
  - Transcription factors

# Space versus analog

- Increased compared to Analog
  - Electron transport
  - Stress
- Mixed
  - Nucleic acid synthesis and modification
  - Heat shock